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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Present Application:

Applicant : Jerry S. Powell

Attorney Docket No. : 500582.12

Filed : Concurrently herewith

Title : HUMAN ERYTHROPOIETIN GENE: HIGH LEVEL EXPRESSION IN STABLY TRANSFECTED MAMMALIAN CELLS

Prior Application:

Examiner : James Martinell

Art Unit : 1633

Serial No. : 08/466,412

PRELIMINARY AMENDMENT &
PETITION FOR EXTENSION OF TIME

Box Patent Application
Commissioner of Patents
Washington, D.C. 20231

Sir:

The continuation application submitted herewith is filed to maintain copendency with the parent application, U.S. Patent Application No. 08/466,412. An Office Action on the parent application was mailed April 11, 2001. Accordingly, Applicant herewith petitions the Commissioner of Patents under 37 C.F.R. § 1.136(a)(3) for a 3–month extension of time from July 11, 2001, to October 11, 2001, to maintain the pendency of the parent application. Submitted herewith is a check in the amount of $1,046, which includes $460 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to Deposit Account No. 50-1266. Please amend the application as follows:

In the Specification:

In accordance with the requirements of 37 C.F.R., § 1.821-1.825, please amend the specification by inserting the enclosed "SEQUENCE LISTING" immediately preceding the claims.

Please replace the Title with the following rewritten Title:
--METHOD OF MAKING GLYCOPROTEIN EXHIBITING ERYTHROPOIESIS REGULATING ACTIVITY AND GLYCOPROTEIN PRODUCED BY THIS METHOD--

Please amend the specification by inserting a new section before the "Technical Field" as follows:
--CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application No. 08/466,412 filed June 6, 1995, which is a continuation of application No. 08/132,489 filed October 6, 1993, now U.S. Patent No 5,688,679, which is a continuation of application No. 07/453,381 filed December 18, 1989, now abandoned, which is a continuation of application No. 07/211,278 filed June 21, 1988, now abandoned, which is a continuation of application No. 06/879,423 filed June 27, 1986, now abandoned.--

Please replace the paragraph beginning at page 2, line 16, with the following rewritten paragraph:

FIGURE 1 is a schematic representation of the subject 2426 bp Apa I restriction fragment that contains the human erythropoietin gene sequences (SEQ ID NO:1).

Please replace the paragraph beginning at page 3, line 13, with the following rewritten paragraph:

Oligonucleotide mixtures were prepared using an Applied Biosystems synthesizer and end-labeled using ^{32}p-ATP and T4 polynucleotide kinase. The synthetic oligonucleotides

were designed to correspond to portions of the amino terminal amino acid sequence (SEQ ID NO:2) of:

H_2N-Ala-Pro-?-Arg-Leu-Ile-Leu-Asp-Ser-Arg-Val-Leu-Glu-Arg-Tyr-Leu-Leu-Glu-Ala-Lys-Glu-Ala-Glu-?-Ile-Thr-Asp-Gly-Gly-Ala
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obtained by Yanagawa et al. (J.Biol.Chem. 259:2707-2710,1984) for the human protein purified from urine of patients with aplastic anemia. To reduce the degeneracy of the codons for the amino acid sequence of this region, the codon usage rules of Grantham et al. (Nucleic Acids Research 8:43-59, 1981) and Jaye et al. (Nucleic Acids Research 11:2325-2335, 1983) were employed. These rules take into account the relatively rare occurrence of CpG dinucleotides in DNA of vertebrates and avoid, where appropriate, potential A:G mismatch pairings. At amino acid position 24, an asparagine was placed as most likely (J.Biol.Chem. 259:2707-2710,1984). For the amino acids Glu-Ala-Lys-Glu-Ala-Glu-Asn (SEQ ID NO:3), 2 pools of 72 sequences each were synthesized to correspond to the predicted codons. Thus, one pool was TT(c/t)TC(a/g/t)GC(c/t)TC(c/t)TT(a/g/t)GCTTC (SEQ ID NO:4) for the 20 nucleotide probe, and the second pool replaced a T with a C at position 18. For the amino acids Glu-Asn-Ile-Thr-Asp-Gly (SEQ ID NO:5), one pool of sequences (AGC TCC TCC ATC AGT ATT ATT T[c/t]) (SEQ ID NO:6) was constructed for the 23 nucleotide probe.

In the Claims:

Please cancel claims 1-9, and add new claims 18-39 as follows:

18. (New) A method of making a glycoprotein exhibiting erythropoiesis regulating activity comprising:

culturing eukaryotic host cells transformed with a DNA construct comprising a eukaryotic promoter sequence operably linked to an insert consisting essentially of the sequence of SEQ ID NO:1 from position 59 through position 2204, the construct also including a 5' untranslated sequence located between the eukaryotic promoter and position 59, the 5'

untranslated sequence being devoid of a sequence encoding a translational initiation codon, the construct also including a 3' untranslated sequence downstream of position 2204, the 3' untranslated region comprising a eukaryotic polyadenylation sequence, where the host cells are cultured under conditions providing for the expression and secretion of the glycoprotein into a culture medium; and

recovering the glycoprotein from the culture medium.

19. (New) The method of claim 18 in which said eukaryotic host cells are baby hamster kidney cells.

20. (New) The method of claim 19 further comprising transforming the host cells with a DNA sequence encoding dihydrofolate reductase and where the host cells are treated with methotrexate prior to recovering the glycoprotein from the culture medium.

21. (New) The method of claim 20 wherein the host cells are treated with a concentration of methotrexate of about 10 μM to about 10 mM; and wherein cells that continue to grow after methotrexate treatment are selected to establish a stable cell culture.

22. (New) The method of claim 21 wherein the host cells are treated with a first concentration of methotrexate of about 1 μM to about 10 mM prior to being treated with a second concentration of methotrexate, the second concentration being lower than the first concentration.

23. (New) The method of claim 22 wherein the second concentration of methotrexate is about 1 μM to about 1 mM.

24. (New) The method of claim 18 wherein the DNA construct includes an adenoviurs major late promoter as the eukaryotic promoter operably linked to the insert.

25. (New) The method of claim 18 wherein the DNA construct includes a metalothionein promoter as the eukaryotic promoter operably linked to the insert.

26. (New) The method of claim 18 wherein titers of said glycoprotein of at least two million units of erythropoietin activity per liter of culture medium are obtained, the units of activity being measured by a radioimmune assay using a mammalian erythropoietin as a standard.

27. (New) A method of making a glycoprotein exhibiting erythropoiesis regulating activity comprising:

culturing eukaryotic host cells transformed with a DNA construct comprising an insert consisting essentially of the sequence according to SEQ ID NO:1 under conditions providing for the expression and secretion of a glycoprotein into the culture medium; and

recovering said glycoprotein from the culture medium.

28. (New) The method of claim 27 wherein the cells are stably transformed and the glycoprotein is produced at a level of about 500 to about 7000 units per ml of culture medium, the units being determined by an in vitro erythroid colony forming bioassay using mouse bone marrow cells and partially purified sheep erythropoietin as a comparative standard.

29. (New) The method of claim 27 wherein the cells are stably transformed and the glycoprotein is produced at a level of about 6 to about 85 μg per ml of culture medium.

30. (New) The method of claim 27 in which said eukaryotic host cells are baby hamster kidney cells.

31. (New) A method of making a glycoprotein exhibiting erythropoiesis regulating activity comprising:

culturing eukaryotic host cells transformed with a DNA construct comprising an insert consisting essentially of the sequence according to SEQ ID NO:1, an adenoviurs-2 major

later promoter sequence, an adenovirus-2 tripartite leader and third leader 5' splice sequence, an immunoglobulin 3' splice sequence and a late SV-40 polyadenylation signal sequence, the insert being operably linked downstream of the adenoviurs-2 major later promoter sequence and upstream of the immunoglobulin 3' splice site to provide for the expression and secretion of the glycoprotein into a culture medium; and

recovering said glycoprotein from the culture medium.

32. (New) The method of claim 29 in which said eukaryotic host cells are baby hamster kidney cells.

33. (New) A method of making a glycoprotein exhibiting erythropoiesis regulating activity comprising:

culturing eukaryotic host cells transformed with a DNA construct comprising an insert consisting essentially of the sequence according to SEQ ID NO:1 and a metalothionein promoter operably linked to the insert to provide expression and secretion of a glycoprotein into the culture medium; and

recovering the glycoprotein from the culture medium.

34. (New) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 10.

35. (New) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 18.

36. (New) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 27.

37. (New) The glycoprotein according to claim 36 wherein the glycoprotein exhibits microheterogeneity in size when analyzed by SDS polyacrylamide gel electrophoresis, and where a first pattern of bands of the glycoprotein detected by Coomassie staining of the SDS

polyacrylamide gel comigrates with a second pattern of bands detected when erythropoietin purified from the urine of a patient with aplastic anemia is analyzed by SDS polyacrylamide gel electrophoresis.

38. (New) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 31.

39. (New) A glycoprotein exhibiting erythropoiesis regulating activity produced by the method of claim 33.

REMARKS

The present amendment restores originally submitted claims 10-17 and adds new claims 18-39 for examination at this time. The submission of this amendment is to pursue certain embodiments of the invention not pursued in the parent applications. Applicant reserves the right to file additional continuation applications to further prosecute various embodiments previously pursued in the parent applications.

All of the claims remaining in the application are now clearly allowable. Favorable consideration and a Notice of Allowance are earnestly solicited.

Respectfully submitted,

DORSEY & WHITNEY LLP

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MWR:sj

Enclosures:
Postcard
Sequence Listing on Paper
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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

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